



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2006

Regular endurance training reduces the exercise induced HIF-1alpha and HIF-2alpha mRNA expression in human skeletal muscle in normoxic conditions

Lundby, Carsten ; Gassmann, Max ; Pilegaard, Henriette

Abstract: Regular exercise induces a variety of adaptive responses that enhance the oxidative and metabolic capacity of human skeletal muscle. Although the physiological adjustments of regular exercise have been known for decades, the underlying mechanisms are still unclear. The hypoxia inducible factors 1 and 2 (HIFs) are clearly related heterodimeric transcription factors that consist of an oxygen-dependent alpha-subunit and a constitutive beta-subunit. With hypoxic exposure, HIF-1alpha and HIF-2alpha protein are stabilized. Upon heterodimerization, HIFs induce the transcription of a variety of genes including erythropoietin (EPO), transferrin and its receptor, as well as vascular endothelial growth factor (VEGF) and its receptor. Considering that several of these genes are also induced with exercise, we tested the hypothesis that the mRNA level of HIF-1alpha and HIF-2alpha subunits increases with a single exercise bout, and that this response is blunted with training. We obtained muscle biopsies from a trained (5 days/week during 4 weeks) and untrained leg from the same human subject before, immediately after, and during the recovery from a 3 h two-legged knee extensor exercise bout, where the two legs exercised at the same absolute workload. In the untrained leg, the exercise bout induced an increase ($P < 0.05$) in HIF-1alpha fold and HIF-2alpha fold mRNA at 6 h of recovery. In contrast, HIF-1alpha and HIF-2alpha mRNA levels were not altered at any time point in the trained leg. Obviously, HIF-1alpha and HIF-2alpha mRNA levels are transiently increased in untrained human skeletal muscle in response to an acute exercise bout, but this response is blunted after exercise training. We propose that HIFs expression is upregulated with exercise and that it may be an important transcription factor that regulates adaptive gene responses to exercise.

DOI: <https://doi.org/10.1007/s00421-005-0085-5>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-110326>

Journal Article

Published Version

Originally published at:

Lundby, Carsten; Gassmann, Max; Pilegaard, Henriette (2006). Regular endurance training reduces the exercise induced HIF-1alpha and HIF-2alpha mRNA expression in human skeletal muscle in normoxic conditions. *European Journal of Applied Physiology*, 96(4):363-369.

DOI: <https://doi.org/10.1007/s00421-005-0085-5>

Carsten Lundby · Max Gassmann · Henriette Pilegaard

Regular endurance training reduces the exercise induced HIF-1 α and HIF-2 α mRNA expression in human skeletal muscle in normoxic conditions

Accepted: 10 October 2005 / Published online: 12 November 2005
© Springer-Verlag 2005

Abstract Regular exercise induces a variety of adaptive responses that enhance the oxidative and metabolic capacity of human skeletal muscle. Although the physiological adjustments of regular exercise have been known for decades, the underlying mechanisms are still unclear. The hypoxia inducible factors 1 and 2 (HIFs) are clearly related heterodimeric transcription factors that consist of an oxygen-dependent α -subunit and a constitutive β -subunit. With hypoxic exposure, HIF-1 α and HIF-2 α protein are stabilized. Upon heterodimerization, HIFs induce the transcription of a variety of genes including erythropoietin (EPO), transferrin and its receptor, as well as vascular endothelial growth factor (VEGF) and its receptor. Considering that several of these genes are also induced with exercise, we tested the hypothesis that the mRNA level of HIF-1 α and HIF-2 α subunits increases with a single exercise bout, and that this response is blunted with training. We obtained muscle biopsies from a trained (5 days/week during 4 weeks) and untrained leg from the same human subject before, immediately after, and during the recovery from a 3 h two-legged knee extensor exercise bout, where the

two legs exercised at the same absolute workload. In the untrained leg, the exercise bout induced an increase ($P < 0.05$) in HIF-1 α fold and HIF-2 α fold mRNA at 6 h of recovery. In contrast, HIF-1 α and HIF-2 α mRNA levels were not altered at any time point in the trained leg. Obviously, HIF-1 α and HIF-2 α mRNA levels are transiently increased in untrained human skeletal muscle in response to an acute exercise bout, but this response is blunted after exercise training. We propose that HIFs expression is upregulated with exercise and that it may be an important transcription factor that regulates adaptive gene responses to exercise.

Introduction

Regular endurance exercise induces a variety of adaptive responses that among others enhance the oxidative capacity and metabolic efficiency of human skeletal muscle. A consistent finding with endurance training is the increase in some of the main metabolism regulating enzymes, i.e. citrate synthase (CS), and lactate dehydrogenase (LDH_{a+b}) and muscle fibre capillarization (Saltin and Gollnick 1983). Although the adaptive responses to regular exercise have been known for many decades, the regulatory mechanisms are still unclear. Our recent work has shown that following a single exercise bout, the mRNA level of several metabolic genes increases transiently, typically peaking a few hours into recovery, and returning to near pre-exercise levels 24 h after the end of exercise (Pilegaard et al. 2000, 2003). It has been proposed, that if the exercise bout is repeated frequently, the mRNA levels of metabolic genes will accumulate and in turn translate into more proteins (Willimas and Neuffer 1996). Our recent findings that PGC-1 α transcription and mRNA content is transiently increased by a single bout of exercise imply that exercise-induced PGC-1 α upregulation could be one important mechanism modulating training-induced mitochondrial biogenesis in human skeletal muscle

While the present manuscript was under revision the paper by Ameln et al. (2005) has been published reporting somewhat contradicting results. Roberto Bottinelli.

C. Lundby · H. Pilegaard
Copenhagen Muscle Research Centre, Copenhagen, Denmark

C. Lundby
Rigshospitalet, Copenhagen, Denmark

M. Gassmann · C. Lundby (✉)
Vetsuisse Faculty and Zürich Center for Integrative Human Physiology (ZIPH), University of Zürich,
Wintherthurerstrasse 260, 8057 Zürich, Switzerland
E-mail: Carsten.Lundby@CMRC.dk

H. Pilegaard
Institute of Molecular Biology and Physiology,
The August Krogh Institute,
University of Copenhagen, Copenhagen, Denmark

(Pilegaard et al. 2003). Another possible candidate involved in the activation of genes that are induced with exercise is the hypoxia inducible factor-1 (HIF-1). HIF-1 is a heterodimeric transcription factor that augments transcription of a variety of genes in response to hypoxia, including erythropoietin (EPO), transferrin (Tf), transferrin receptor (TfR), hexokinase I and II (HKI and HKII), phosphofructokinase (PFK-L), vascular endothelial growth factor (VEGF), VEGF receptor FLT-1, and lactate dehydrogenase (LDH_a), many of which are also augmented at the protein level with regular exercise training. The biological activity of HIF-1 is determined by the activity of its α -subunits (HIF-1 α and HIF-2 α), which are both increased with hypoxia in most cells [revised in Hopfl et al. (2003, 2004)]. HIF-1 α is known to increase in hypoxic rat skeletal muscle (Stroka et al. 2001) whereas HIF-2 α is uninvestigated in mammalian skeletal muscle. At present it is unknown as whether HIF-1 α and/or HIF-2 α expression is regulated by exercise alone in the absence of additional exposure to ambient hypoxia. If so, this will suggest that HIF-1 could be one of the important transcription factors controlling at least some of the adaptive responses for exercise in human skeletal muscle.

The purpose of the present study was to test the hypothesis that a single prolonged exercise bout increases HIF-1 α and/or HIF-2 α mRNA levels in the untrained leg while the expression of both HIFs should be reduced in the trained leg.

Methods

Subjects

Seven healthy, normally physically active subjects not involved in any type of endurance activities participated in the present study. The subjects ranged in age from 19 to 24 years and had an average height of 184 ± 3 cm (mean \pm SE) and a mean weight of 84 ± 7 kg. After being given both written and oral information on the experimental protocol and procedures, the subjects gave their informed, written consent to participate. The study conformed with guidelines laid down in the Declaration of Helsinki and was approved by the Copenhagen and Frederiksberg Ethics Committee, Denmark.

Experimental design

A modified bicycle ergometer (Andersen and Saltin 1985) was applied for both one-legged and two-legged knee extensor exercise. Before initiating the training period, each of the subjects completed a one-legged knee extensor exercise performance test with each leg separately to determine the maximal load that could be maintained for 2 min (2 min max). The leg to be trained was chosen randomly. The subjects trained 5 days/week

for 4 weeks by one-legged knee extensor exercise. The first training session was performed at 70% of 2 min max until exhaustion (about 1 h). As performance was increased over time, the resistance was increased keeping the duration approximately the same.

The subjects were provided with a standardized dinner and evening snack meal the day before the experiments. On the day of experiments, the subjects arrived at the laboratory after consuming a light breakfast. A resting muscle biopsy was taken (about 2.5 h after breakfast) from the middle portion of the vastus lateralis muscle of each leg using the percutaneous needle biopsy technique with suction (Bergström 1962), and immediately frozen in liquid nitrogen and stored at -80°C . The subjects performed two-legged knee extensor exercise for 3 h at the one-legged 2 min max of the untrained leg (i.e. per leg, 50% of the 2 min max of the untrained leg). Additional biopsies were obtained from both legs at the end of exercise and after 2, 6, and 24 h of recovery. Food intake was restricted throughout the experiment until after the 6 h biopsy after which the subjects were provided with standardized meals including dinner and breakfast the next morning (2.5 h before the 24 h biopsy). For a more detailed description see Pilegaard et al. (2003).

RNA isolation and reverse transcription

Total RNA was isolated from about 20 mg of tissue by a modified guanidinium thiocyanate–phenol–chloroform extraction method adapted from Chomczynski and Sacchi (1987) as described previously (Pilegaard et al. 2000). The reverse transcription reaction was performed by using the Superscript II Rnase H⁻ system and oligo (dT) primers (Invitrogen, Carlsbad, CA, USA), as described previously (Pilegaard et al. 2000).

PCR

HIF-1 α and HIF-2 α mRNA was quantified by fluorescence-based real-time PCR (ABI PRISM 7900 Sequence Detection System, Applied Biosystems). Forward (FP) and reverse (RP) primers and TaqMan probes were designed from human specific sequence data (Entrez-*NIH* and *Ensembl*, Sanger Institute) using computer software (Primer Express, Applied Biosystems). One oligonucleotide from each set was designed to span an exon–exon junction, eliminating the possibility of amplifying genomic DNA. The following sequences were used to amplify a fragment of HIF-1 α FP: 5' GCCCCAGATTCAGGATCAGA 3'; RP: 5' TGG GACTATTAG-GCTCAGGTGAAC 3'; probe: 5' ACCTAGTCCTTCCGATGGAAGCACTAGACAA 3', and of HIF-2 α FP: 5' GCCACCCAGTACCAGGACT ACA 3'; RP: 5' CCTCACAGTCATATCTGGTCAG TTCG 3'; probe 5' TCAGCCCACAAGGTGTCAGG CATG 3'. The probes were 5' 6-carboxyfluorescein

(FAM) and 3' 6-carboxy-*N,N,N',N'*-tetramethylrhodamine (TAMRA) labelled. Prior optimization was conducted determining optimal primer concentrations, probe concentration and verifying the efficiency of the amplification. The expected size of the PCR product was confirmed on a DNA 2.5% agarose gel. GAPDH was amplified for use as endogenous control using a pre-developed assay reagent (Applied Biosystems). As reported previously, GAPDH levels were not influenced by either the exercise protocol or the training (Pilegaard et al. 2003), and has recently been validated for this type of studies (Lundby et al. 2005).

PCR amplification was performed (in triplicate) in a total reaction volume of 10 μ l. The reaction mixture consisted of 1 μ l diluted template, forward and reverse primers and probe as determined from the prior optimization, 2X TaqMan Universal MasterMix optimized for TaqMan reactions (Applied Biosystems; containing AmpliTaq Gold DNA polymerase, AmpErase Uracil *N*-glycosylase, dNTPs with dUTP, ROX as passive reference and buffer components), and nuclease-free water. The following cycle profile was used for all genes: 50°C for 2 min + 95°C for 10 min + [95°C for 15 s + 60°C for 1 min] \times 40 cycles. A serial dilution from a representative sample was made samples and these samples were amplified together with the unknown samples and used to construct a standard curve from which the threshold cycle numbers of the unknown samples were converted to relative amounts.

Statistics

The mRNA contents were normalized to GAPDH mRNA levels. For statistical analysis, ratios were log transformed and two-way ANOVA for repeated measures was used to evaluate the effect of time and training state on the response of mRNA content and one-way ANOVA for repeated measures was used to evaluate the effect of time for each leg separately. Student–Newman–Keul post hoc test was used to locate differences. A paired *t* test was used to compare the pre-exercise mRNA content in the untrained and trained legs. Differences were considered significant at $P < 0.05$. Samples were expressed relative to pre-sample in the untrained leg, which was set to one. Values in figures are mean \pm SD.

Results

Performance

After training, knee extensor exercise at the load used in the first session (70% of 2 min max) could easily be sustained for more than 100 min with the trained leg (before training, exhaustion occurred after approximately 60 min). Average time to exhaustion in an all-out bout at about 110% of the 2 min max resistance of the

untrained leg was significantly improved by training (3.6 ± 0.7 min for the untrained and 7.2 ± 1.7 min for trained).

Content of mRNA (Fig. 1)

Skeletal muscle biopsies were taken from the untrained and the trained leg before (pre), immediately after prolonged exercise (0 h) as well as after 2, 6, and 24 h of recovery. In the untrained leg, the exercise bout induced an increase ($P < 0.05$) in HIF-1 α and HIF-2 α mRNA at 6 h of recovery relative to before exercise. Compared with rest, the peak increase was approximately tenfold for HIF-1 α , and fivefold for HIF-2 α and the inductions were no longer apparent after 24 h of recovery. In contrast, in the trained leg, there were no exercise-induced transient increases in either HIF-1 α or HIF-2 α mRNA during recovery from the prolonged exercise bout.

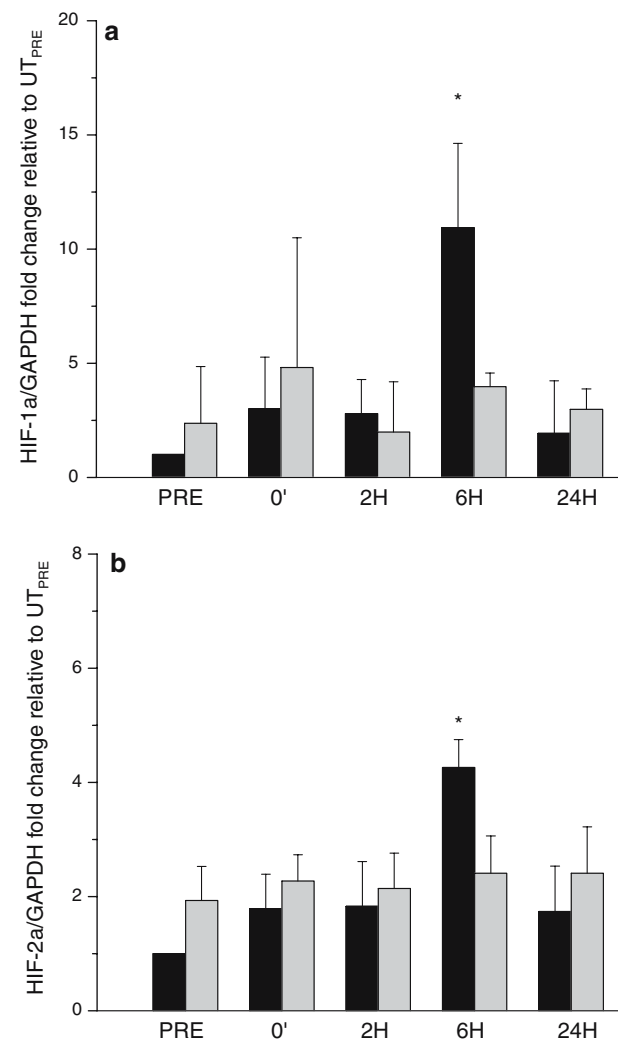


Fig. 1 HIF-1 α (a) and HIF-2 α (b) mRNA in the untrained (black) and trained (grey) leg following 3 h of 2-legged kicking exercise

Discussion

The present investigation tested the hypothesis that exercise increases the mRNA expression of HIF-1 α and HIF-2 α and that previous regular endurance training reduces this response. This was tested by obtaining biopsies from the vastus lateralis muscle before and at the end of 3 h of exercise as well as during 24 h of recovery in a previously trained and an untrained leg from the same subject. The main findings are that (a) in the untrained leg, a 3 h knee extensor exercise bout increased HIF-1 α and HIF-2 α mRNA transiently, while (b) an exercise-induced increase was not present in the trained leg. These observations show that the mRNA expression of HIF-1 α and HIF-2 α is increased with acute exercise, and imply that upregulation of HIF-1 α and HIF-2 α might be important in regulating the early adaptive response to endurance training.

Choice of exercise intensity

The quadriceps training induced increases in peak work rate of 24% (Mourtzakis et al. 2004), and the relative load on the trained versus the untrained leg was therefore also different during the 3 h two-legged exercise. The subjects performed two-legged knee extensor exercise for 3 h at 87 ± 5 W, and as the two legs performed similar amount of work (Pilegaard et al. 2003), i.e. approximately 43.5 W per leg, then the relative exercise intensity for the untrained leg was 54 and 44% for the trained leg. This difference in intensity may have influenced the results, as it is well known that the choice of exercise intensity among others influences the contribution of aerobic versus anaerobic metabolism and fuel utilization. However, the differences in exercise intensities are small and may be of minor relevance. An advantage of choosing the two-legged kicking ergometer for this study is that O₂ and substrate availability as well as hormones to the exercising legs are equal.

HIF-1 α and HIF-2 α presence in muscle tissue

The presence of HIF-1 α mRNA and protein in animal muscle tissue is well known (Wiener et al. 1996; Stroka et al. 2001), and also human muscle tissue has previously been shown to contain HIF-1 α mRNA (Vogt et al. 2001). The distribution of HIF-2 α mRNA has, however, only recently been investigated in various rat tissues (Wiesener et al. 2003) and the authors of that study concluded that HIF-2 α mRNA is widespread in microvascular endothelial cells (Wiesener et al. 2003), and is thus likely also present within human muscle. Accordingly, the present study is the first to show the presence of HIF-2 α mRNA in human skeletal muscle.

HIF-1 α and HIF-2 α regulation in muscle tissue

The main trigger for HIF accumulation is hypoxia. Under normoxic conditions HIF-1 α has been shown to be rapidly ubiquitinated and subjected to proteasomal degradation (Jewell et al. 2001). With hypoxia however, HIF is stabilized and accumulates in the nucleus [reviewed in Hopfl et al. (2004)]. Thus, in exercise, the easiest answer to how HIF-1 is induced would be tissue hypoxia. However, whether tissue hypoxia is present as a consequence of exercise is debated. For many years the consensus was that intracellular PO₂ (PiO₂) declines as exercise intensity increases (Connett et al. 1984; Gladden 1996). Most of the earlier reports on reduced PiO₂ with exercise were based on concomitant increments in blood lactate, and thus assumed anaerobiosis to be a result of lowered PiO₂. More recent measurements of PiO₂ assessed by myoglobin desaturation by MRS technology have revealed that (1) at the onset of graded exercise PiO₂ is reduced in some subjects, but not in all, and (2) beyond 50–60% of maximum work the variability in PiO₂ is greatly reduced and falls to a relatively uniform low level of about 4 mmHg. As with the early lactate measurements, this suggests that PiO₂ does not decrease linearly with increasing metabolic rate (Richardson et al. 2001). Although PiO₂ appears to decline with increasing work rates, this does not necessarily impair the oxygen gradient from blood to cell. At rest mean capillary PO₂ is about 40 mmHg (Richardson et al. 1999a) and PiO₂ is around 8 mmHg (Mole et al. 1999) equivalent to a pressure gradient of 32 mmHg. At maximum exercise, end capillary PO₂ declines to approximately 35 mmHg (Richardson et al. 1999a), and PiO₂ to 4 mmHg (Richardson et al. 1995), which equals a pressure gradient of 31 mmHg. Thus, despite PiO₂ is halved in the transition from rest to maximum work rates, the pressure gradient seems relatively unaffected, and may thus also leave oxygen conductance unchanged. Interestingly, Honing's group suggested in the early 1990s that the distribution of oxygen within the muscle might be more uniform with exercise than at rest by increasing flow to less perfused areas, and thus 'protecting' against regional tissue hypoxia (Honing et al. 1991). In contrast to the reduced PiO₂ this would imply an even better oxygenated muscle with exercise compared with the resting muscle.

The HIF α mRNA induction in the present exercise studies does not seem to depend on tissue hypoxia. Firstly, the induction of HIF-1 α and HIF-2 α mRNA occurred 6 h into recovery where muscle hypoxia must be assumed to be non-existing and thereby unable to induce the changes. Secondly, it also seems unlikely that there should be differences in oxygenation levels at rest between a trained and untrained muscle, and alternative mechanisms appear to explain the exercise-induced augmentations in HIF-1 α and HIF-2 α mRNA. Therefore, the induction signal should be sought for elsewhere. HIF-1 mRNA expression can be augmented by other factors than tissue hypoxia, and includes interleukin-1 β (IL-1 β), insulin like growth factor (IGF) I and

II, insulin, heregulin, epidermal growth factor, TNF α , angiotensin-2 (Feldser et al. 1999; Hellwig-Burgel et al. 1999; Laughner et al. 2001; Zelzer et al. 1998; Zhong et al. 2000; Richard et al. 2000; Gorchach et al. 2001), and also nitric oxide (NO) has been shown to be a molecule capable of inducing HIF-1. Of these TNF- α , IL-1, and insulin are known to be increased only little after a single prolonged exercise bout (Rennie et al. 1976; Ostrowski et al. 1999), whereas IGF-1 (Ehrnborg et al. 2003) and NO (Joyner and Tschakovsky 2003) are elevated more markedly. With the present studies it is however impossible to determine if any of these molecules are involved in the exercise-induced increases in HIF-1 α or HIF-2 α mRNA. However, the present findings show for the first time that exercise in normoxic conditions can elevate HIF-1 α and HIF-2 α mRNA during recovery suggesting that exercise in itself may elicit regulation of the HIF α genes in human skeletal muscle. One previous study has investigated the effects of a single exercise bout on HIF-1 α mRNA content. In that study a muscle biopsy was obtained 30 min into recovery after a 45 min one-legged kicking exercise regime, and revealed no increases in HIF-1 α mRNA (Gustafsson et al. 1999). In the present study, we report a transient increase in HIF-1 α /HIF-2 α mRNA after an acute 3 h exercise bout at ~50% of maximum capacity exercise in normoxic conditions. Specifically, HIF-1 α /HIF-2 α was unchanged immediately at termination of exercise and also after 2 h into recovery and thus in accordance with the previous findings (Gustafsson et al. 1999). However, after 6 h of recovery HIF-1 α /HIF-2 α mRNA was increased approximately tenfold and fivefold, respectively, compared with prior to exercise, and declined back to pre-exercise values after 24 h of recovery. These observations lead to the question what could be the stimulus associated with exercise eliciting the HIF gene responses, and what could be the functional role of such exercise-induced increases in HIF protein.

Overall, the effect of increased HIFs is to increase oxygen delivery and the metabolic potential of the cell, and similar adaptive responses are known to occur with regular exercise training, and a possible role for HIFs to increase after a single exercise bout could be to activate the transcription of target genes. The specific DNA sequence to which HIFs binds is referred to as hypoxic response element (HRE) and is located in promotor or enhancer sequences of HIFs target genes, where binding leads to upregulation of that specific gene. Target genes in various tissues include EPO, Tf, TfR, HKI and HKII, PFK-L, VEGF, VEGF receptor FLT-1, heme oxygenase (HO)-1, and the LDH α gene. Of these the expression of several is augmented with acute and/or regular exercise training (Saltin and Gollnick 1983; Richardson et al. 1999b; Koval et al. 1998; Pilegaard et al. 2000). The increases in HIF-1 α /HIF-2 α mRNA in the untrained leg during recovery in the present study were associated with a similar time course of HKII mRNA induction (Pilegaard et al. 2003), whereas total VEGF mRNA was augmented immediately at termination of exercise and

remained elevated until 6 h of recovery in the untrained leg. Although the present data suggest that transcriptional regulation of HIFs is not a major mechanism controlling the VEGF gene response to prolonged exercise, this does not rule out that regulation of HIFs protein stability could be important in inducing the VEGF gene in response to exercise; and obviously, other factors than HIFs could be regulating the exercise-induced responses of VEGF. Such a possible VEGF induction by the exercise-induced increases in HIF-1 α /HIF-2 α , may result in an increased capillarization, which will allow the mean transient time of the red blood cell to decrease when passing the muscle capillaries. In turn, this increases the time available for O $_2$ and metabolite extraction. Another exercise related advantage associated with genes activated by HIF-1 α /HIF-2 α is the potential increase in glycolytic flux within the mitochondria.

HIF-1 α /HIF-2 α mRNA and chronic exercise training

The effect of exercise training on HIF-1 α mRNA content in human skeletal muscle has been investigated in two previous studies (Vogt et al. 2001; Ookawara et al. 2002). In agreement with the present study, an intensive training regime lasting 3 months (Ookawara et al. 2002) has been reported not to affect the resting HIF-1 α mRNA content 48 h after the last training bout. In addition, the observations by Vogt et al. (2001) that HIF-1 α mRNA was increased by hypoxic training, but not by normoxic training, in muscle biopsies obtained 24 h after the last exercise stimulus are also in line with the present finding that the HIF-1 α mRNA levels were similar in the untrained and trained muscle 24 h after the acute exercise bout. The lack of training-induced increases in resting HIF-1 α mRNA (Vogt et al. 2001; Ookawara et al. 2002) (and present study) and HIF-2 α mRNA (present study) supports the present observations that the HIF α mRNA levels returned to pre-exercise values at 24 h of recovery, demonstrating that due to the time course of the HIF α mRNA responses to exercise, training-induced accumulations of HIF-1 α and HIF-2 α mRNA are not to be expected with this type of training stimulus.

Conclusion

The main conclusion of the present study is that HIF-1 α and HIF-2 α mRNA is increased transiently with a single bout of endurance exercise, and that this response is blunted after a previous 4 week training period when the exercise is performed at the same absolute exercise intensity. These observations imply that the mRNA expression of HIFs is increased with acute exercise, and thereby may be an important transcription factor involved in the adaptive response to endurance training.

Acknowledgments Lara Ogunshola, Stephan Keller, and Bengt Saltin are thanked for their fruitful discussions throughout the study and while preparing the manuscript. Max Gassmann is founded by the Swiss National Science Foundation. Henriette Pilegaard and Carsten Lundby is founded by The Danish Medical Research Council and The Danish Natural Science Research Council.

References

- Ameln H, Gustafsson T, Sundberg CJ, Okamoto K, Jansson E, Poellinger L, Makino Y (2005) Physiological activation of hypoxia inducible factor-1 in human skeletal muscle. *FASEB J* 19:1009–1011
- Andersen P, Saltin B (1985) Maximal perfusion of skeletal muscle in man. *J Physiol* 366:233–249
- Bergström J (1962) Muscle electrolytes in man. *Scan J Clin Lab Invest* 68:1–110
- Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal Biochem* 162:156–159
- Connert RJ, Gayeski TE, Honig CR (1984) Lactate accumulation in fully aerobic, working, dog gracilis muscle. *Am J Physiol* 246:H120–H128
- Ehrnborg C, Lange KH, Dall R, Christiansen JS, Lundberg PA, Baxter RC, Boroujerdi MA, Bengtsson BA, Healey ML, Pentecost C, Longobardi S, Napoli R, Rosen T (2003) The growth hormone/insulin-like growth factor-I axis hormones and bone markers in elite athletes in response to a maximum exercise test. *J Clin Endocrinol Metab* 88:394–401
- Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, Semenza GL (1999) Reciprocal positive regulation of hypoxia-inducible factor 1 α and insulin-like growth factor 2. *Cancer Res* 59:3915–3918
- Gladden L (1996) Lactate transport and exchange during exercise. In: *Handbook of physiology. Exercise: regulation and integration of multiple systems*. Am Physiol Soc, Bethesda, pp 614–649
- Gorlach A, Diebold I, Schini-Kerth VB, Berchner-Pfannschmidt U, Roth U, Brandes RP, Kietzmann T, Busse R (2001) Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: role of the p22(phox)-containing NADPH oxidase. *Circ Res* 89:47–54
- Gustafsson T, Puntchart A, Kaijser L, Jansson E, Sundberg CJ (1999) Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am J Physiol* 276:H679–H685
- Hellwig-Burgel T, Rutkowski K, Metzen E, Fandrey J, Jelkmann W (1999) Interleukin-1 β and tumor necrosis factor- α stimulate DNA binding of hypoxia-inducible factor-1. *Blood* 94:1561–1567
- Honig CR, Gayeski TE, Clark A Jr, Clark PA (1991) Arteriovenous oxygen diffusion shunt is negligible in resting and working gracilis muscles. *Am J Physiol* 261:H2031–H2043
- Hopfl G, Ogunshola O, Gassmann M (2003) Hypoxia and high altitude. The molecular response. *Adv Exp Med Biol* 543:89–115
- Hopfl G, Ogunshola O, Gassmann M (2004) HIFs and tumors—causes and consequences. *Am J Physiol Regul Integr Comp Physiol* 286:R608–R623
- Jewell UR, Kvietikova I, Scheid A, Bauer C, Wenger RH, Gassmann M (2001) Induction of HIF-1 α in response to hypoxia is instantaneous. *FASEB J* 15:1312–1314
- Joyner MJ, Tschakovsky ME (2003) Nitric oxide and physiologic vasodilation in human limbs: where do we go from here? *Can J Appl Physiol* 28:475–490
- Koval JA, DeFronzo RA, O'Doherty RM, Printz R, Ardehali H, Granner DK, Mandarino LJ (1998) Regulation of hexokinase II activity and expression in human muscle by moderate exercise. *Am J Physiol* 274:E304–E308
- Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL (2001) HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1 α (HIF-1 α) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* 21:3995–4004
- Lundby C, Nordsborg N, Kusuhabara K, Kristensen KM, Neufer PD, Pilegaard H (2005) Gene expression in human skeletal muscle: alternative normalization method and effect of repeated biopsies. *Eur J Appl Physiol* 1–10
- Mole PA, Chung Y, Tran TK, Sailasuta N, Hurd R, Jue T (1999) Myoglobin desaturation with exercise intensity in human gastrocnemius muscle. *Am J Physiol* 277:R173–R180
- Mourtzakis M, Gonzalez-Alonso J, Graham TE, Saltin B (2004) Hemodynamics and O₂ uptake during maximal knee extensor exercise in untrained and trained human quadriceps muscle: effects of hyperoxia. *J Appl Physiol* 97:1796–1802
- Ookawara T, Suzuk K, Haga S, Ha S, Chung KS, Toshinai K, Hamaoka T, Katsumura T, Takemasa T, Mizuno M, Hitomi Y, Kizaki T, Suzuki K, Ohno H (2002) Transcription regulation of gene expression in human skeletal muscle in response to endurance training. *Res Commun Mol Pathol Pharmacol* 111:41–54
- Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK (1999) Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol* 515(Pt 1):287–291
- Pilegaard H, Ordway GA, Saltin B, Neufer PD (2000) Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *Am J Physiol Endocrinol Metab* 279:E806–E814
- Pilegaard H, Saltin B, Neufer PD (2003) Exercise induces transient transcriptional activation of the PGC-1 α gene in human skeletal muscle. *J Physiol* 546:851–858
- Rennie MJ, Park DM, Sulaiman WR (1976) Uptake and release of hormones and metabolites by tissues of exercising leg in man. *Am J Physiol* 231:967–973
- Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD (1995) Myoglobin O₂ desaturation during exercise. Evidence of limited O₂ transport. *J Clin Invest* 96:1916–1926
- Richardson RS, Leigh JS, Wagner PD, Noyszewski EA (1999a) Cellular PO₂ as a determinant of maximal mitochondrial O₂ consumption in trained human skeletal muscle. *J Appl Physiol* 87:325–331
- Richardson RS, Wagner H, Mudaliar SR, Henry R, Noyszewski EA, Wagner PD (1999b) Human VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. *Am J Physiol* 277:H2247–H2252
- Richard DE, Berra E, Pouyssegur J (2000) Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1 α in vascular smooth muscle cells. *J Biol Chem* 275:26765–26771
- Richardson RS, Newcomer SC, Noyszewski EA (2001) Skeletal muscle intracellular PO₂ assessed by myoglobin desaturation: response to graded exercise. *J Appl Physiol* 91:2679–2685
- Saltin B, Gollnick PD (1983) Skeletal muscle adaptability: significance for metabolism and performance. In: *Handbook of physiology. Exercise: regulation and integration of multiple systems*, Am Physiol Soc, Bethesda, pp 555–631
- Stroka DM, Burkhardt T, Desbaillets I, Wenger RH, Neil DA, Bauer C, Gassmann M, Candinas D (2001) HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. *FASEB J* 15:2445–2453
- Vogt M, Puntchart A, Geiser J, Zuleger C, Billeter R, Hoppeler H (2001) Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *J Appl Physiol* 91:173–182
- Wiener CM, Booth G, Semenza GL (1996) In vivo expression of mRNAs encoding hypoxia-inducible factor 1. *Biochem Biophys Res Commun* 225:485–488
- Wiesener MS, Jürgensen JS, Rosenberger C, Scholze CK, Horstrup JH, Warnecke C, Mandriota S, Bechmann I, Frei UA, Pugh CW, Ratcliffe PJ, Bachmann S, Maxwell PH, Eckardt KU

- (2003) Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *FASEB J* 17:271–273
- Willimas RS, Neuffer PD (1996) Regulation of gene expression in skeletal muscle by contractile activity. In: Rowell LSJ (ed) *The handbook of physiology. Exercise: regulation, integration of multiple systems*. Oxford University Press, New York, pp 1124–1150
- Zelzer E, Levy Y, Kahana C, Shilo BZ, Rubinstein M, Cohen B (1998) Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT. *EMBO J* 17:5085–5094
- Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL (2000) Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* 60:1541–1545